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## Supplementary Material

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### Vitamin D receptor inhibits NLRP3 activation by impeding Its

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### BRCC3-mediated deubiquitination

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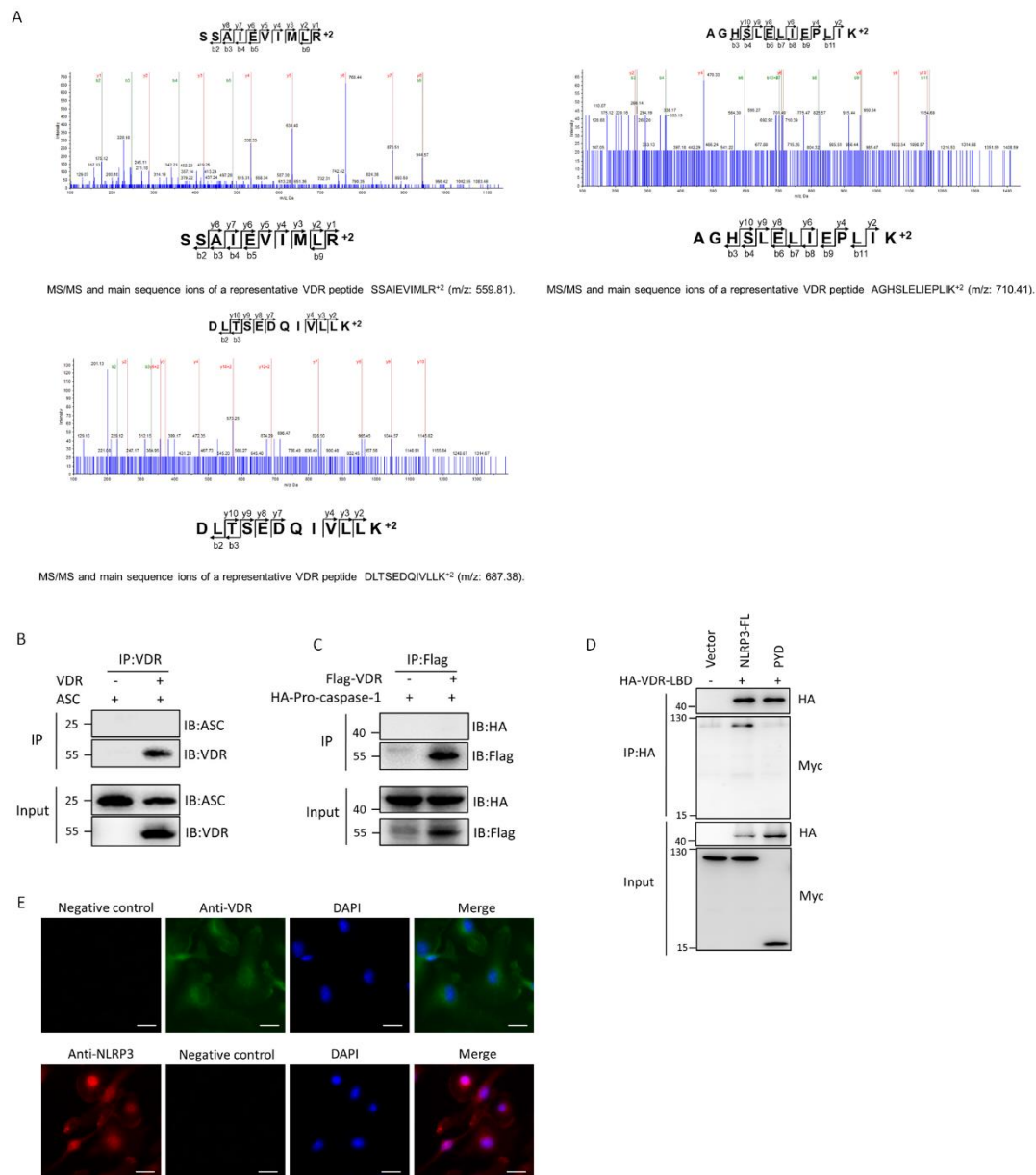
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### FIGURE S1. VDR does not interact with ASC or pro-caspase-1, related to Figure

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(A) Mass spectrometry analysis of NLRP3 with Flag to pull down NLRP3-associated

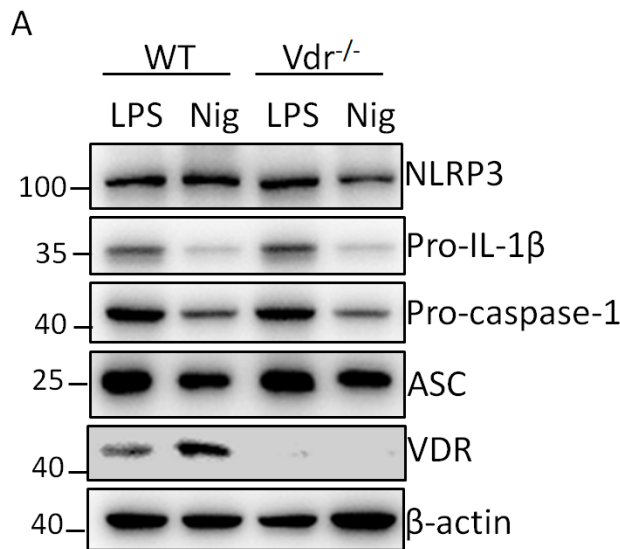
12 proteins in Flag-NLRP3-overexpressing HEK293T cells. MS/MS map and main  
13 sequence ions of a representative VDR peptide.

14 (B) Flag-VDR was co-expressed with HA-tagged ASC in HEK293T cells, and proteins  
15 were immunoprecipitated and analysed by immunoblotting. Whole-cell lysates are  
16 shown as the input.

17 (C) Flag-VDR was co-expressed with HA-tagged pro-caspase-1 in HEK293T cells, and  
18 proteins were immunoprecipitated and analysed by immunoblotting. Whole-cell lysates  
19 are shown as the input.

20 (D) Wild-type or mutant NLRP3 (PYD) and HA-VDR-LBD were expressed in  
21 HEK293T cells, immunoprecipitated and analysed by immunoblotting.

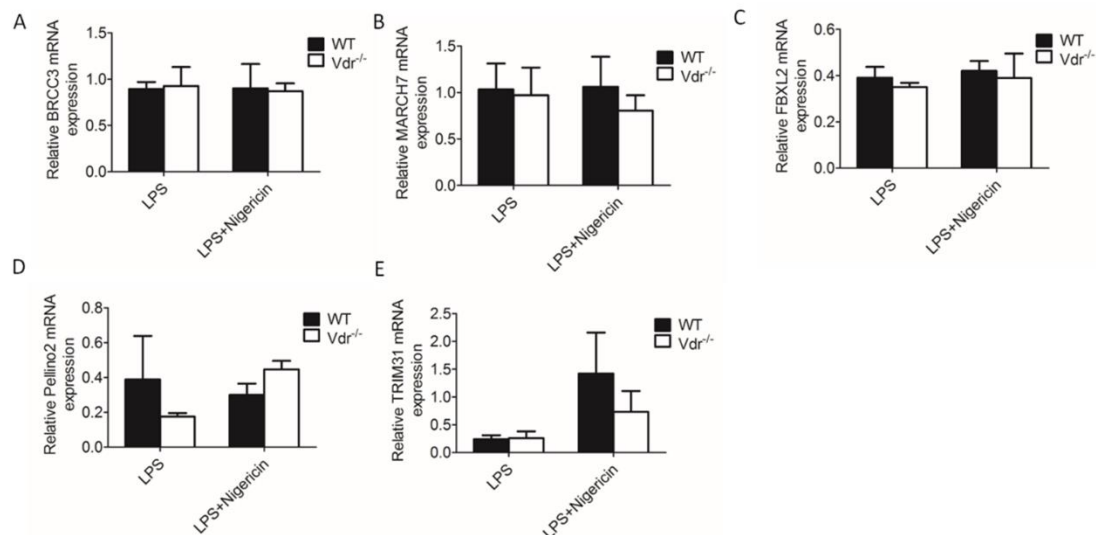
22 (E) Negative control for anti-VDR and anti-NLRP3 in Immunofluorescence with  
23 isotype antibody. The cells were stained with isotype antibody and second antibody, the  
24 nuclei were visualized with DAPI staining. Scale bar, 20  $\mu$ m.



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26 **FIGURE S2. NLRP3, ASC, pro-IL-1β and pro-caspase-1 protein expression in**  
27 **BMDMs (wild-type and Vdr<sup>-/-</sup>): Related to Figure 2**

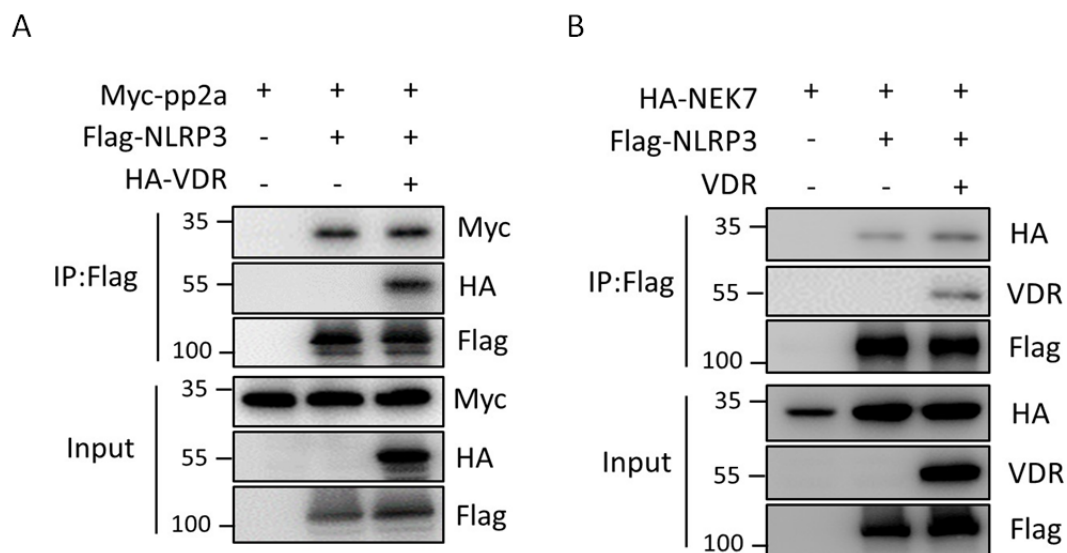
28 (A) LPS-primed BMDMs (wild-type and Vdr<sup>-/-</sup>) were stimulated with nigericin. The  
29 protein levels of NLRP3, ASC, pro-IL-1β and pro-caspase-1 in whole-cell lysates were  
30 analysed by immunoblotting.



**FIGURE S3.VDR mediating NLRP3 ubiquitining does not depend on transcription regulating: related to Figure 4**

The mRNA expression of NLRP3 related deubiquitinase (BRCC3) (A) and ubiquitinase (MARCH7,FBXL2,Pellino2,TRIM31) (B-E) in LPS primed BMDMs (wild-type and *Vdr*<sup>-/-</sup>) with or without Nigericin.

Data are representative of three independent experiments.



**FIGURE S4. Vitamin D receptor does not interfere with the interaction between NEK7, PP2A and NLRP3: related to Figure 4**

(A-B) HEK293T cells were transfected with indicated vectors. Immunoprecipitated with anti-Flag and analyzed by immunoblotting.